Published in final edited form as:

Pediatr Blood Cancer. 2008 February ; 50(2): 274–279. doi:10.1002/pbc.21213.

Correlation between Asparaginase Sensitivity and Asparagine Synthetase Protein Content, but not mRNA, in Acute Lymphoblastic Leukemia Cell Lines

Nan Su, BS¹, Yuan-Xiang Pan, PhD¹, Mi Zhou, MD², Richard C. Harvey, MD³, Stephen P. Hunger, MD², Michael S. Kilberg, PhD^{1,*}

¹Department of Biochemistry and Molecular Biology, University of Florida Shands Cancer Center, University of Florida College of Medicine, Gainesville, Florida

²Department of Pediatrics, University of Florida Shands Cancer Center, University of Florida College of Medicine, Gainesville, Florida

³Department of Pathology, University of New Mexico School of Medicine, Albuquerque, New Mexico

Abstract

Background.—Asparaginase (ASNase) is an essential component of most treatment protocols for childhood acute lymphoblastic leukemia (ALL). Although increased asparagine synthetase (ASNS) expression may contribute to ASNase resistance, there is conflicting data from patient samples with regard to correlation between ASNS mRNA content and ASNase sensitivity.

Procedure.—Both T-cell and B-cell derived ALL cell lines were treated with ASNase and then monitored for cell proliferation, cell death, and ASNS mRNA and protein expression.

Results.—Despite elevated ASNS mRNA following ASNase treatment, different ALL cell lines varied widely in translation to ASNS protein. Although ASNS mRNA levels did not consistently reflect ASNase sensitivity, there was an inverse correlation between ASNS protein and ASNase-induced cell death. Expression of ASNS in an ASNase-sensitive cell line resulted in enhanced ASNase resistance, and conversely, siRNA-mediated inhibition of ASNS expression promoted increased drug sensitivity.

Conclusion.—These observations provide an explanation for the ASNase sensitivity of ALL cells and demonstrate the importance of measuring ASNS protein rather than mRNA in predicting ASNase responsiveness.

Keywords

amino acids; starvation; apoptosis; nutrient control; cancer; chemotherapy

*Correspondence to: Michael S. Kilberg, Department of Biochemistry and Molecular Biology, University of Florida College of Medicine, Box 100245, JHMC, Gainesville, Florida 32610-0245. mkilberg@ufl.edu.

This article contains Supplementary Material available at <http://www.interscience.wiley.com/jpages/1545-5009/suppmat>.

INTRODUCTION

L-Asparaginase (ASNase) is an important chemotherapeutic for childhood acute lymphoblastic leukemia (ALL) and clinical trials have shown that intensified ASNase treatment can improve outcome [1,2]. ASNase administration leads to depletion of plasma asparagine and a subsequent loss of intracellular asparagine [3]. Most tissues contain sufficient asparagine synthetase (ASNS) activity to maintain asparagine or the enzyme is up-regulated in response to the asparagine depletion [4,5]. Primary ALL cells and many ALL cell lines exhibit a particularly low level of ASNS expression [6,7], and therefore, are unusually sensitive to asparagine depletion. Drug-selected ASNase-resistant ALL cell lines exhibit elevated expression of ASNS [8,9], and over-expression of exogenous ASNS protein results in an ASNase-resistant phenotype in the absence of drug selection [9].

Despite the ability of primary ALL cells to up-regulate ASNS mRNA content in response to ASNase treatment in vitro [10] or in vivo [11], asparagine remains an essential amino acid for this cell population. This apparent paradox makes it difficult to explain the sensitivity of ALL cells to ASNase treatment. Studies have documented that in vitro ASNase sensitivity of ALL blasts is correlated with in vivo drug resistance and prognosis [12–15]. However, published data correlating ASNS protein or activity and ASNase resistance in human cells is limited. In a 1969 study, Haskell and Canellos [16] observed higher ASNS enzymatic activity in five ASNase-resistant versus four drug-sensitive patients. More recently, Dübbers et al. [17] reported similar values of ASNS activity in lymphoblasts from children with AML or ALL, but there was a large range in the activity levels among the patients. By microarray analysis, Fine et al. [18] observed a correlation between ASNS mRNA content and ASNase sensitivity in 16 ALL cell lines, but no strong correlation in primary ALL cells.

Stams et al. [10] hypothesized that inhibition of ASNS expression by the TEL-AML1 protein might explain the increased in vitro sensitivity to ASNase of TEL-AML1(+) ALL cells, but surprisingly, their study showed that TEL-AML1(+) ALL blasts expressed five-fold more ASNS mRNA than did TEL-AML1(–) cells or normal controls. They also observed no difference in the capacity to up-regulate ASNS mRNA after exposure to ASNase. Krejci et al. [19] observed increased ASNS mRNA in TEL-AML1(+) cells, despite increased ASNase sensitivity relative to TEL-AML1(–) cells. Interestingly, although 10 of 15 TEL-AML1(+) patients with ASNS mRNA below the median relapsed, none of the patients with elevated ASNS relapsed. However, Stams et al. [20] did not see a difference in relapse relative to ASNS mRNA levels. Holleman et al. [21] tested the ASNase sensitivity of blasts from 173 patients and correlated sensitivity with ASNS mRNA determined by oligonucleotide arrays. While ASNS was not one of the top 35 genes associated with in vitro ASNase resistance, ASNS mRNA levels were 2.9 times higher ($P < 0.001$) in patients classified as ASNase resistant. These results are consistent with an inverse relationship between ASNS mRNA and ASNase sensitivity in 60 human cancer cell lines [22].

A caveat to many of these recent studies is that ASNS mRNA was analyzed, not protein content or enzymatic activity. The conflicting published data underscore the need to better understand the relationship between ASNS expression and ASNase sensitivity. The present studies were designed to test the correspondence between ASNS mRNA expression and

ASNS protein content and to determine which of these parameters better correlates with ASNase sensitivity.

MATERIALS AND METHODS

Cell Culture

The T-ALL cell line MOLT-4 has been used to investigate the metabolic and gene expression changes following ASNase treatment [9,23,24]. To extend these studies to B-lineage ALL lines, TEL-AML1(-) NALM-6 and TEL-AML1(+) REH cell lines were chosen for comparison to the parental MOLT-4 cells (MOLT-4P), a drug-selected ASNase resistant subclone MOLT-4R, and MOLT-4P/ASNS, a MOLT-4P subclone stably-transfected with ASNS [9,23,24]. The cells were propagated in RPMI-1640 (Cellgro) medium [9] and the MOLT-4R cells were maintained in 1 IU/ml of ELSPAR ASNase (MERCK). Leukemic blasts, isolated from ALL children at the time of diagnosis, were obtained from the Children's Oncology Group (COG). These specimens were entered into the COG ALL cell bank with informed consent of the patient/parent. The Institutional Review Board of the University of Florida approved the use of these specimens.

Trypan Blue Exclusion and WST-1 Cell Proliferation Assays

Staining with 0.2% trypan blue solution and counting replicate samples on a hemocytometer established cell number and viability. The proliferation of ALL cells was determined by a 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfohenyl)-2H-tetrazolium (WST-1) assay (Roche, Indianapolis, IN). Proliferation curves were plotted as the optical density readings versus the ASNase concentration. Each of the cell lines was treated with 0–5 IU/ml ASNase for 72 hr. A single, three-parameter exponential decay curve was fit to the data points and used to estimate an IC₅₀ (Sigma Plot, Systat Software Inc.).

RNA Isolation and Quantitative Real Time RT-PCR

ASNS mRNA was determined by real-time quantitative RT-PCR (qPCR) [4]. Amplification primers were: ASNS, sense 5'-GCAGCTGAAAGAAGCCCAAGT-3' and anti-sense 5'-TGTCTTCCATGCCAATTGCA-3'. For an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measured using primers: sense 5'-TTGGTATCGTGGAAGGACTC-3' and anti-sense 5'-ACAGTCTTCTGGGTGGCAGT-3'. Samples from at least three independent experiments, each measured in duplicate, were analyzed with data expressed as the means±standard error of the means.

Immunoblotting

Protein extracts were subjected to immunoblotting, as described previously [25], using a monoclonal antibody against human ASNS [23]. The blots were reprobed with anti-actin antibody, as a control.

RESULTS

Sensitivity to ASNase

Analysis of total viable cell number and percent viability following exposure to 1 IU/ml ASNase confirmed the sensitivity of MOLT-4P cells and the insensitivity of the drug-selected ASNase-resistant MOLT-4R counterpart (Fig. 1). Neither of the B-cell lines, NALM-6 and REH, exhibited substantial cell death, but growth was slower than that of MOLT-4R illustrating the cytostatic nature of ASNase for these particular cell lines. MOLT-4P/ASNS cells, that over-express exogenous ASNS, showed a significant decrease in ASNase sensitivity compared to MOLT-P, confirming that elevated ASNS expression alone is sufficient to induce a more drug-resistant phenotype [9].

Concentration Dependence of ASNase Action

To determine the relative sensitivity of the five cell lines, cells were incubated for 72 hr in 0–5 IU/ml ASNase and then the cell proliferation and viability were analyzed. The initial cell number was 5×10^4 for each cell line, so in Supplemental Figure S1 the value at 0 IU/ml represents their relative growth rates over the 72 hr, which were: MOLT-4R and NALM-6 > MOLT-4P and MOLT-4P/ASNS >> REH. Although NALM-6 and REH were not as sensitive as MOLT-4P, their growth was suppressed by ASNase levels greater than 1 IU/ml. When the percent viability was analyzed, the relative ASNase sensitivities were readily apparent also (Fig. 2). Whereas there was less than 20% cell death in MOLT-4R at 5 IU/ml ASNase, there was about 50% killing in the NALM-6 and REH populations. MOLT-4P was dramatically more sensitive to ASNase than all other cell lines with an estimated IC_{50} of 0.004 IU/ml (Fig. 2). Conversely, MOLT-4R has an IC_{50} of greater than 25 IU/ml ASNase. The NALM-6, REH, and MOLT-4P/ASNS were intermediate in their sensitivity with approximate IC_{50} values of 6.1, 3.5, and 0.7 IU/ml, respectively.

ASNS Expression after Acute ASNase Treatment

Data from MOLT-4P cells [9,23] and primary blasts [11] indicated that ASNS mRNA levels are increased in response to ASNase, but ASNS protein content has not been reported. To assess ASNS mRNA and protein expression, the five cell lines were exposed to 1 IU/ml ASNase for 0–48 h (Fig. 3A and B). In response to ASNase treatment, the ASNS mRNA content of all five cell lines increased by 12 hr, and remained elevated for the entire 48 hr period (Fig. 3A). The basal ASNS protein content varied greatly among the cell lines (Fig. 3B). MOLT-4P cells had an extremely low level, whereas the MOLT-4R cells contained the greatest amount of ASNS protein and the NALM-6, REH, and MOLT-4P/ASNS were intermediate. In sharp contrast to the changes in ASNS mRNA, only the MOLT-P cells exhibited a time-dependent increase in ASNS protein content in response to ASNase treatment (Fig. 3C). However, the degree of increase relative to basal (T=0 hr) did not reach statistical significance until 24 hr (Fig. 3C). The highest MOLT-P value (15.7 ± 4.7 at 48 hr) was still less than the lowest basal value (REH cells, 20.3 ± 4.6) for the remaining cell types. To illustrate the relationship between ASNase sensitivity and ASNS expression, the estimated IC_{50} values for the three parental ALL cell lines were compared to ASNS mRNA and protein values (Fig. 3D). The correlation between ASNS protein and IC_{50} was much greater than that for the IC_{50} versus ASNS mRNA content. These results are consistent with

the observations described below showing that ASNS protein levels are extremely low in most newly diagnosed patients (Fig. 4).

Inhibition of ASNS Expression Enhances ASNase Sensitivity

Given that lymphocytes, including ALL cell lines, are difficult to transfect, HepG2 human hepatoma cells were transfected with a siRNA against ASNS to test the hypothesis that reducing ASNS expression in an ASNase resistant cell would increase the drug sensitivity. HepG2 cells were transduced with a retrovirus containing a short hairpin RNA (siRNA) targeting nt +620 to +640 of the ASNS mRNA [9]. The transduced HepG2 cells were then challenged with 2 IU/ml ASNase for 48 hr. Apoptosis analysis was performed by flow cytometry using propidium iodide and annexin V-FITC staining [9]. HepG2 cells, which exhibit high levels of ASNS protein and an estimated IC₅₀ of greater than 5 IU/ml ASNase, were transfected with a control or an ASNS-specific siRNA and then subjected to an ASNase challenge (2 IU/ml) (Supplemental Figure S2). There was a minimal increase in apoptosis after delivery of the control siRNA, but the dependence of cell viability on ASNS was illustrated by the reduction of cell viability from 78% to 47% by the ASNS siRNA. Furthermore, cells treated with both ASNase and the ASNS-specific siRNA exhibited a decrease in viability to 21% of the total population.

ASNS mRNA and Protein Expression in Patient Samples

ASNS mRNA levels were monitored in 40 ALL diagnostic specimens, including 29 cases of B-precursor ALL (1 infant, 7 standard-risk, 21 high-risk), 6 cases of T-ALL, and 5 not further characterized. Six of 29 B-precursor ALL specimens were TEL-AML1-positive and 23 were negative. All of the patients had a very low level of ASNS mRNA, none more than three-fold higher than that expressed in MOLT-4P, and all were substantially less than levels present in the other cell lines (data not shown). ASNS protein levels were measured in a subset of these patient samples that represented both high and low values of ASNS mRNA content. Immunoblotting revealed that ASNS protein was undetectable in 9 of 10 samples (Fig. 4). In the single exception (sample #3; a TEL-AML1-negative high-risk B-precursor ALL case), the ASNS protein abundance was greater than that in MOLT-4P, but much lower than the MOLT-4R cells. The ASNS mRNA content of sample #3 was the fifth highest of the 40 specimens analyzed.

DISCUSSION

MOLT-4P cells express ASNS protein at extremely low levels, but increasing ASNS expression, by ASNase-selection (MOLT-4R) or forced over-expression (MOLT-4P/ASNS), induces ASNase resistance [9]. The current experiments extend those observations and include the following novel observations. (1) The B-ALL cell lines REH and NALM-6 express a basal level of ASNS mRNA and protein that is greater than MOLT-4P, but less than drug-selected MOLT-4R cells. (2) ASNase action was primarily cytostatic, against REH, NALM-6, and MOLT-4P/ASNS; only MOLT-4P cells exhibited a significant amount of cell death. (3) The ASNase sensitivity of the two B-ALL cell lines was similar despite the fact that REH are TEL-AML1(+) and NALM-6 are not. (4) For the three parental cell lines, ASNase sensitivity did not correlate with the ASNS mRNA level, but it did

with ASNS protein content. (5) There are cell-specific differences in ASNS protein content that cannot be predicted from the mRNA level. (6) ASNase induced ASNS mRNA in all five cells, but only in the MOLT-4P cells was the ASNS protein increased significantly. However, the up-regulated MOLT-4P ASNS protein content was still less than the basal level in the ASNase-insensitive cell populations. (7) While ALL patient samples, from time of initial diagnosis, expressed variable amounts of ASNS mRNA, the levels were similar in magnitude to those in ASNase-sensitive MOLT4-P cells. Only one of ten specimens expressed ASNS protein at a level above that observed in MOLT4-P, and that level was orders of magnitude lower than those present in the highly resistant MOLT4-R line. Taken together, these data suggest that ASNase resistance due to a high level of ASNS protein expression is, at most, infrequent at the time of initial diagnosis. (8) Inhibition of ASNS expression enhances ASNase sensitivity.

ASNS mRNA analysis has led to variable conclusions regarding the relationship between ASNS mRNA levels in primary ALL cells and ASNase sensitivity. Holleman et al. [21] reported a significantly higher level of ASNS mRNA in blasts of patients who were resistant to ASNase in vitro, whereas others have not observed this correlation [11,18,26]. Those differing results are consistent with the present data documenting that neither ASNS protein expression nor ASNase sensitivity necessarily correlate with ASNS mRNA content. In contrast, ASNase sensitivity was inversely related to ASNS protein abundance. Given that most patients are initially ASNase sensitive, their cells are likely reflected by the drug-sensitive MOLT-4P cells. The ASNS protein in 10 newly diagnosed ALL patients was similar to that observed in MOLT-4P cells. The ASNase sensitivity of ALL patients may be the consequence of the following two observations. First, in MOLT-4P there was a delay of 12–24 hr between the initial exposure to ASNase and the increase in ASNS protein. Second, even after ASNase induction, the MOLT-4P ASNS protein content was still less than the basal level in the ASNase-resistant ALL cell lines. Obviously, extensive analysis of patient samples will be necessary to determine if these results in cell culture have applicability to therapy. This caution is underscored by the results of Dübbers et al. [17] who observed that ASNS enzymatic activity varied up to 50-fold among ALL patients.

TEL-AML1(+) ALL patients are more sensitive to ASNase [10,27], despite the fact that ASNS mRNA content in these patients can equal that in the TEL-AML1(–) patients [10,26]. ASNS protein content has not been monitored in these populations. The TEL-AML1(–) NALM-6 cells and the TEL-AML1(+) REH cells were not substantially different in their change in ASNS mRNA content after ASNase exposure, but, consistent with the estimated IC₅₀ values reported here and with observations in patients [10], the basal level of ASNS protein was twice as great in the NALM-6 cells compared to the REH cells.

Collectively, the present results demonstrate that measurement of ASNS protein, rather than mRNA, may serve as a better indicator of ASNase sensitivity, potentially explaining the published inconsistencies between ASNS mRNA and drug sensitivity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENT

This research was supported by grants to MSK from the Institute of Diabetes, Digestive and Kidney Diseases (DK-52064) and to SPH from the National Cancer Institute (CA-107437). The authors wish to thank the other members of the Kilberg and Hunger laboratories for helpful discussion. Patient samples were provided by the Children's Oncology Group ALL Cell Bank, which was supported by National Cancer Institute grants CA98543 and CA114766.

Abbreviations:

AML	acute myelogenous leukemia
ALL	acute lymphoblastic leukemia
ASNS	asparagine synthetase
ASNase	asparaginase
FACS	fluorescence activated cell sorting
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
WST-1	4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate

REFERENCES

1. Sallan SE, Hitchcock-Bryan S, Gelber R, et al. Influence of intensive asparaginase in the treatment of childhood non-T-cell acute lymphoblastic leukemia. *Cancer Res* 1983;43:5601–5607. [PubMed: 6352020]
2. Amylon MD, Shuster J, Pullen J, et al. Intensive high-dose asparaginase consolidation improves survival for pediatric patients with T cell acute lymphoblastic leukemia and advanced stage lymphoblastic lymphoma: a Pediatric Oncology Group study. *Leukemia* 1999;13:335–342. [PubMed: 10086723]
3. Cooney DA, Capizzi RL, Handshumacher RE. Evaluation of L-asparagine metabolism in animals and man. *Cancer Res* 1970;30: 929–935.
4. Chen H, Pan YX, Dudenhausen EE, et al. Amino acid deprivation induces the transcription rate of the human asparagine synthetase gene through a timed program of expression and promoter binding of nutrient-responsive bZIP transcription factors as well as localized histone acetylation. *J Biol Chem* 2004;279:50829–50839. [PubMed: 15385533]
5. Kilberg MS, Pan YX, Chen H, et al. Nutritional control of gene expression: how mammalian cells respond to amino acid limitation. *Annu Rev Nutr* 2005;25:59–85. [PubMed: 16011459]
6. Asselin BL, Kurtzburg J. Asparaginase. In: Pui C-H, editor. *Treatment of acute leukemias*. Totawa, NJ: Humana Press; 2003. 365–379.
7. Rizzari C. Asparaginase treatment. In: Pui C-H, editor. *Treatment of acute leukemias*. Towata, NJ: Humana Press; 2003. 381–391.
8. Den Boer ML, Pieters R, Kazemier KM, et al. Relationship between major vault protein/lung resistance protein, multidrug resistance-associated protein, P-glycoprotein expression, and drug resistance in childhood leukemia. *Blood* 1998;91:2092–2098. [PubMed: 9490695]
9. Aslanian AM, Fletcher BS, Kilberg MS. Asparagine synthetase expression alone is sufficient to induce L-asparaginase resistance in MOLT-4 human leukaemia cells. *Biochem J* 2001;357:321–328. [PubMed: 11415466]
10. Stams WA, den Boer ML, Beverloo HB, et al. Sensitivity to L-asparaginase is not associated with expression levels of asparagine synthetase in t(12;21)+ pediatric ALL. *Blood* 2003; 101:2743–2747. [PubMed: 12433682]

11. Appel IM, den Boer ML, Meijerink JP, et al. Upregulation of asparagine synthetase expression is not linked to the clinical response to L-asparaginase in pediatric acute lymphoblastic leukemia. *Blood* 2006;107:4244–4249. [PubMed: 16497975]
12. Pieters R, Klumper E, Kaspers GJL, et al. Everything you always wanted to know about cellular drug resistance in childhood acute lymphoblastic leukemia. *Crit Rev Oncol Hematol* 1997;25:11–26. [PubMed: 9134309]
13. Kaspers GJL, Veerman AJP, Pieters R, et al. In vitro cellular drug resistance and prognosis in newly diagnosed childhood acute lymphoblastic leukemia. *Blood* 1997;90:2723–2729. [PubMed: 9326239]
14. Kaspers GJL, Pieters R, Van Zantwijk CH, et al. Prednisone resistance in childhood acute lymphoblastic leukemia: vitro–vivo correlations and cross-resistance to other drugs. *Blood* 1998;92: 259–266. [PubMed: 9639525]
15. Asselin BL, Ryan D, Frantz CN, et al. *In vitro* and *in vivo* killing of acute lymphoblastic leukemia cells by L-asparaginase. *Cancer Res* 1989;49:4363–4368. [PubMed: 2743326]
16. Haskell CM, Canellos GP. L-asparaginase resistance in human leukaemia–asparagine synthetase. *Biochem Pharm* 1969;18: 2578–2580. [PubMed: 4935103]
17. Dubbers A, Wurthwein G, Muller HJ, et al. Asparagine synthetase activity in paediatric acute leukaemias: AML-M5 subtype shows lowest activity. *Br J Haematol* 2000;109:427–429. [PubMed: 10848836]
18. Fine BM, Kaspers GJ, Ho M, et al. A genome-wide view of the in vitro response to L-asparaginase in acute lymphoblastic leukemia. *Cancer Res* 2005;65:291–299. [PubMed: 15665306]
19. Krejci O, Starkova J, Otova B, et al. Upregulation of asparagine synthetase fails to avert cell cycle arrest induced by L-asparaginase in TEL/AML1-positive leukaemic cells. *Leukemia* 2004;18:434–441. [PubMed: 14724653]
20. Stams WA, den Boer ML, Beverloo HB, et al. Upregulation of asparagine synthetase and cell cycle arrest in t(12;21)-positive ALL. *Leukemia* 2004;19:318–319.
21. Holleman A, Cheok MH, den Boer ML, et al. Gene-expression patterns in drug-resistant acute lymphoblastic leukemia cells and response to treatment. *N Engl J Med* 2004;351:533–542. [PubMed: 15295046]
22. Scherf U, Ross DT, Waltham M, et al. A gene expression database for the molecular pharmacology of cancer. *Nat Genet* 2000; 24:236–244. [PubMed: 10700175]
23. Hutson RG, Kitoh T, Amador DAM, et al. Amino acid control of asparagine synthetase: relation to asparaginase resistance in human leukemia cells. *Am J Physiol* 1997;272:C1691–C1699. [PubMed: 9176161]
24. Aslanian AM, Kilberg MS. Multiple adaptive mechanisms affect asparagine synthetase substrate availability in asparaginase resistant MOLT-4 human leukemia cells. *Biochem J* 2001; 358: 59–67. [PubMed: 11485552]
25. Leung-Pineda V, Pan Y, Chen H, et al. Induction of p21 and p27 expression by amino acid deprivation of HepG2 human hepatoma cells involves mRNA stabilization. *Biochem J* 2004;379:79–88. [PubMed: 14715082]
26. Stams WA, den Boer ML, Holleman A, et al. Asparagine synthetase expression is linked with L-asparaginase resistance in TEL-AML1 negative, but not in TEL-AML1 positive pediatric acute lymphoblastic leukemia. *Blood* 2005;11:4223–4225.
27. Ramakers-van Woerden NL, Pieters R, Loonen AH. TEL/AML1 gene fusion is related to in vitro drug sensitivity for L-asparaginase in childhood acute lymphoblastic leukemia. *Blood* 2000;96:1094–1099. [PubMed: 10910927]

Fig. 1.

ALL cell lines show differential growth patterns and sensitivities to ASNase treatment. The five ALL-derived cell lines were diluted to 50×10^4 cells/ml and treated with 1 IU/ml of ASNase for the indicated times. The cells were given fresh medium after 36 hr of treatment. Total viable cells (top panel) or percent cell viabilities (bottom panel) were plotted versus time of ASNase treatment. The results shown are the averages $\pm$ standard deviations from three separate experiments.

Fig. 2.

Effect of ASNase concentration on cell death of ALL cell lines. ALL cells were diluted to $50 \times 10^4 \text{ ml}^{-1}$ and then a 100 μl aliquot placed in each well of a 96-well plate. After treatment with ASNase for 72 hr, the percent cell viability was determined. The results shown are the averages $\pm$ standard deviations from three separate experiments, and for clarity, data from treatments with less than 0.1 IU/ml of ASNase are shown as inserts.

Fig. 3.

ASNS mRNA and protein expression in ALL cell lines during ASNase treatment. ALL cells were diluted to 50×10^4 cells/ml and treated with 1 IU/ml of ASNase for the indicated times. The medium was changed at 36 hr post-treatment. (panel A) RNA was collected at each time point and subjected to quantitative RT-PCR using primers specific for ASNS and GAPDH. ASNS mRNA levels were normalized to the GAPDH level and the fold induction relative to MOLT-4P cells at time 0 were plotted. (Panels B and C) Whole cell extracts were collected and subjected to immunoblotting for ASNS or actin (panel B) and the results were quantified by densitometry (panels B and C). In panel B, the immunoblots and the quantified data, normalized to actin levels, are shown for the basal amount of ASNS protein (time=0 hr). In panel C, the data are plotted relative to the value for MOLT4-P cells at time=0 hr. The results shown are the averages+standard deviations from three separate experiments, and those values that are significantly different ($P < 0.05$) from the corresponding control

are indicated with an asterisk. Panel D shows the relationship between ASNase IC₅₀ and ASNS protein or mRNA expression. For the three parental cell lines (MOLT-4P, NALM-6, and REH), the estimated IC₅₀ values for ASNase sensitivity from the data of Figure 2 were compared to the relative amount of ASNS protein (upper) or ASNS mRNA (lower) shown in panels A and C.

Fig. 4.

ASNS protein content in childhood ALL primary patient samples. Whole cell extracts were prepared from ALL patient samples obtained at the time of initial diagnosis. The samples (15 μ g/lane) were analyzed by immunoblotting for either ASNS or actin protein levels. As a reference for relative abundance, cell extract from MOLT-4P and MOLT-4R cells was also included.